Corrections for Applicant

SUMMARY OF THE INVENTION

According to one aspect of the invention, there is provided a composition comprising a polypeptide in crystalline form, wherein the polypeptide is a TNF-α-converting enzyme polypeptide. In one embodiment, the TNF-α-converting enzyme polypeptide comprises the TNF-α-converting enzyme catalytic domain. In another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the pro and catalytic domains of TNF-α-converting enzyme. In a further embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the amino acid residues 1-477 of TNF-α-converting enzyme. In yet another embodiment, the polynucleotide is substituted such that amino acid residue Ser266 is changed to Ala and amino acid residue Asn542 is changed to Gln, and wherein a second polynucleotide encoding the sequence Gly-Ser-$(His)_6$ is fused to the C-terminus.

(SEQ ID NO:8)

SEQ ID NO:8

typo?

According to another aspect of the invention, the compositions above further comprising a binding partner suitable for co-crystallization with the TNF-α-converting enzyme polypeptide. In one embodiment, the binding partner is a hydroxamate-based binding partner. In another embodiment, the binding partner is N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine,2-(amino)ethyl amide.

According to further embodiments, the compositions above have a crystal structure diffracting to 2.0 Å, are monoclinic, have a unit cell comprising four crystallographically independent TNF-α-converting enzyme catalytic domain (TCD) molecules, have the TCD molecules are in an asymmetric unit, and/or have monoclinic space group $P2_1$ and the cell has the constants a=61.38 A, b=126.27 A, c=81.27 A, and β=107.41°.

embodiment, the coordinates are the coordinates of Table 1, or a substantial part thereof. In a further embodiment, the TNF-α-converting enzyme polypeptide crystal comprises the TNF-α-converting enzyme catalytic domain. In still another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the pro and catalytic domains of TNF-α-converting enzyme. In yet another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the amino acid residues 1-477 of TNF-α-converting enzyme. In another embodiment, the polynucleotide is substituted such that amino acid residue Ser266 is changed to Ala and amino acid residue Asn542 is changed to Gln, and wherein a second polynucleotide encoding the sequence Gly-Ser-$(His)_6$ is fused to the C-terminus. In a further embodiment, the TNF-α-converting enzyme polypeptide crystal is co-crystallized with a binding partner. In still another embodiment, the binding partner is a hydroxamate-based binding partner or N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine,2-(amino)ethyl amide. In yet other embodiments, the TNF-α-converting enzyme polypeptide crystal has a crystal structure diffracting to 2.0 Å, is monoclinic, has a unit cell comprising four crystallographically independent TNF-α-converting enzyme catalytic domain (TCD) molecules, has the TCD molecules are in an asymmetric unit, and/or is of monoclinic space group $P2_1$ and the cell has the constants a=61.38 Å, b=126.27 Å, c=81.27 Å, and β=107.41°. In still another embodiment, the invention the the associating compound is designed to associate with the S1' region of TNF-α-converting enzyme. In yet another embodiment, the associating compound is designed to associate with the S1'S3' pocket of TNF-α-converting enzyme. In still other embodiments of the invention, the associating compound is designed to (i) incorporate a moiety that chelates zinc, (ii) form a hydrogen bond with Leu348 or Gly349 of TNF-α-converting enzyme, (iii) introduce a non-polar group which occupies the S1' pocket of TNF-α-converting enzyme, (iv) introduce a group which

lies within the channel joining S1' - S3' pockets of TNF-α-converting enzyme and which makes appropriate van der Waal contact with the channel, and/or (v) form a hydrogen bond with Leu348 or Gly349 on the backbone amide groups of TNF-α-converting enzyme. (SEQ ID NO: 8)

These and other aspects of the invention will become apparent to the skilled artisan in view of the teachings contained herein.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1: Figure 1 is a ribbon diagram of the TACE catalytic domain (TCD). The chain starts on the lower left back side, runs through the structural elements sI, hAI, hA, sII, hB, hB2, sIII, IV, IVa, sIVb, sV, hC, Met-turn and hD, and ends in the upper left back. The three disulfides are shown as connections, with the sulphurs given as small spheres. The catalytic zinc (central sphere) is liganded by the three imidazoles of His405, His409 and His415 (of SEQ ID NO: 8), and by the hydroxyl and the carbonyl oxygen atoms of the inhibitor hydroxamic acid group. The inhibitor mimicking interaction of primed-site residues of a peptide substrate is shown in full. Figure 1 was made using SETOR. See Evans, S. "SETOR: Hardware Lighted Three-Dimensional Solid Model Representations of Macromolecules" *J. Mol. Graph.* 11:134-138 (1993).

Figs. 2a and 2b: Figures 2a and 2b are solid surface representations of the catalytic domains of TACE (TCD) (Figure 2a) and MMP-3 (Figure 2b). The electrostatic surface potential is contoured from -15 (intense red) to 15 (intense blue) k_BT/e. Both active-site clefts run from left to right, with the catalytic zinc atoms (spheres) in the centers. In TACE, the bound inhibitor is shown in full structure, binding with its isobutyl (P1') and its Ala (P3') sidechains into the deep S1' and the novel S3' pockets. The orientation is similar to Fig. 1. Figures 2a and 2b were

made using GRASP. Nicolls, A., Bharadwaj, R. and Houig, B., "Grasp - Graphical representation and analysis of surface properties," *Biophys.* 64, A166 (1993).

Fig. 3: Figure 3 aligns the catalytic domain sequences of adamalysin II (ADAM_CROAD), TACE and human ADAM 10 (hADAM10), according to their topological equivalence and sequence similarity, respectively. The residue numbers are due to the generic TACE numbering. Arrows and braces represent β-strands and α-helices in TACE.

Fig. 4: Figure 4 is a stereo section of the final 2.0 Å electron density around the catalytic zinc (large, central sphere) superimposed with the final TACE model. Visible are the three zinc liganding imidazole rings of His405 (top), His409 (left) and His415 (bottom), the "catalytic" Glu406, and the hydroxamic acid moiety of the inhibitor.. The orientation is similar to Fig. 1. Figure 4 was made using TURBO-FRODO. See Roussel, A. & Cambilleau, C., "Turbo-Frodo in Silicon Graphics Geometry," *Partners Directory*, Silicon Graphics, Mountain View, CA (1989).

Fig. 5: Figure 5 is a superposition of the ribbon plots of the catalytic domain of TACE (light) and adamalysin (dark). Also shown is the catalytic zinc of TACE (sphere) and the three (TACE) and two (adamalysin) disulfide bridges. The orientation is similar to Fig. 1. Figure 5 was made using GRASP.

Fig. 6: Figure 6 illustrates a system for studying a TNF-α converting enzyme, including a video memory storing information for generating a visual display of at least a portion of a TNF-α converting enzyme.

The TACE amino acid sequence, or any part or residue thereof, can be found in Black *et al.*, "A Metalloproteinase disintigrin that releases tumour-necrosis factor-α from cells," *Nature* 385: 729-733 (Feb. 1997), herein incorporated in the entirety by reference. Variations in the amino acid sequence of TACE are within the present invention as well. All references to the TACE amino acid sequence contained herein refer to the sequence in Black *et al.*, supra.

As used herein, the TACE catalytic domain (TCD) refers to the portion of a TACE polypeptide between residues 215 and 477 of SEQ ID NO:8 and including the preceding furin cleavage site (residues 211-214), or any part thereof that is capable of cleaving the peptide PLAQAVRSSS (corrected is substitute spec)

Expression, Isolation and Purification of TACE Polypeptides

Tumor necrosis factor-α converting enzyme (TACE) is described in the published PCT application No. WO 96/41624. The application describes isolated nucleic acids encoding TACE or portions of TACE, expression vectors comprising a cDNA encoding TACE or portions thereof, and host cells transformed or transfected with the expression vectors comprising a cDNA encoding TACE or portions of TACE. The application further describes processes for producing TACE and portions thereof, for example by culturing transfected cells engineered to express TACE, followed by purification of the recombinantly produced TACE or portion thereof. Methods of isolating, expressing, and purifying a TACE polypeptide are described in detail in published PCT application No. WO 96/41624. The entirety of PCT 96/41624 is incorporated herein by reference.

According to the invention, cDNA encoding the signal peptide, pro and catalytic domains of TACE, *i.e.*, amino acid residues 1-477 is inserted into a suitable expression vector and expressed in a suitable cell line. The cDNA also may include other regions that facilitate expression or achieve other objects that

otherwise that do not depart from the essence of the invention, such as flanking regions.

The cDNAs encoding the TACE polypeptide, or functional portions thereof, such as the TCD, may be altered by addition, substitution, deletion, or insertion. Such alterations may be made, for example, to prevent glycosolation, prevent formation of incorrect or undesired disulfide bridges, and/or enhance expression. Examples of such alterations are described in WO 96/41624 and can be carried out by the methods described therein and other conventional methods. TACE may also be conjugated. Such conjugates may comprise peptides added to facilitate purification and/or identification. Such peptides include, for example, poly-His peptides. Conjugation is described in U.S. Patent No. 5,011,912 and Hopp *et al.*, *Bio/Technology* 6:1204 (1988).

In one embodiment of the invention, the cDNA encodes a TNF-α converting enzyme polypeptide comprising the signal peptide, pro and catalytic domains of TACE (TCD), residues 1-477 (SEQ ID NO:8), with Ser266 changed to Ala and Asn452 changed to Gln. These substitutions are useful in preventing N-linked glycosolation. Additionally, the sequence Gly-Ser$(His)_6$ may be added to the C-terminus. The addition of the sequence Gly-Ser$(His)_6$ facilitates purification of the polypeptide using metal-chelate affinity resins, such as Ni-NTA resins.

Recombinant expression vectors containing the nucleotide sequence encoding TACE, or a portion thereof, may be prepared using well known methods. Suitable host cells for expression of TACE polypeptides include prokaryotic, yeast, and higher eukaryotic cells. Vectors and host cells suitable for use in the present invention are described in WO 96/41624. Further examples of suitable expression systems that can be employed to express recombinant TACE according to the present invention include mammalian or insect host cell culture expression systems, including baculovirus systems in insect cells (See Luckow and Summers, *Bio/Technology* 6:47 (1988))and mammalian cell lines such as COS-7 cells

(Gluzman et al., *Cell* 23:175 (1981)). Additional examples are known in the art and include those described in WO 96/41624. In one embodiment of the invention, the TACE polypeptide is expressed in CHO cells. In this embodiment, the cells secrete a mixture of TACE polypeptide beginning with Val212 and Arg215A of SEQ ID NO:8.

In one embodiment, stable expressing cells may be selected by culturing the cells in a drug that kills those cells that do not incorporate the vector. Examples of suitable selection methods are described in, for example, Kaufman, R.J., "Selection and coamplification of heterologous genes in mammalian cells," *Methods in Enzymology*, 185:537-566 (1990).

Purification of the expressed TACE polypeptide may be carried out by any suitable means, such as those described in WO 96/41624. According to one aspect of the invention, it is preferable to obtain a TACE polypeptide that is suitable for crystallization. In obtaining a TACE polypeptide suitable for crystallization, it is important that the process for purifying the TACE polypeptide is sufficient to yield a polypeptide pure enough to properly crystallize.

A preferred method of purification starts with a suitable amount of medium from the culture of TACE-secreting cells. This medium is generally a supernate of the culture. The medium contains the TACE polypeptide to be purified. Preferably, the TACE polypeptide is recombinantly produced using DNA coding for the TACE polypeptide with the sequence altered to encode a conjugate or conjugates that facilitate purification. For example, the sequence encoding Gly-Ser-$(His)_6$ may be added to the C-terminus to facilitate purification using metal-chelate resins.

The medium is concentrated, for example, by diafiltration. Suitable diafiltration units include a Millipore 10K cut-off, 1 ft^2 TFF diafiltration unit. A suitable buffer solution is then added to the concentrated medium. Any suitable buffer may be used. One such suitable buffer contains 20 mM Tris (pH 7.5) and 300 mM NaCl.

TACE Crystal and Methods of Crystallization of TACE Polypeptides

One aspect of the invention relates to a method of crystallizing a TACE polypeptide. A preferred method comprises co-crystallizing a TACE polypeptide with a binding partner described above. Exemplary means for obtaining the TACE polypeptide, as well as purification of the polypeptide are described above.

Crystals may be grown or formed by any suitable method, including drop vapor diffusion, batch, liquid bridge, and dialysis, and under any suitable conditions. Crystallization by drop vapor diffusion is often preferable. In addition, those of skill in the art will appreciate that the crystallization conditions may be varied. Various methods of crystallizing polypeptides are generally known in the art. See, for example, WO 95/35367, WO 97/15588, EP 646 599 A2, GB 2 306 961 A, and WO 97/08300.

In one embodiment of the invention, a DNA construct comprising TACE residues 1-477 (SEQ ID NO:8), with Ser266 changed to Ala, Asn452 changed to Gln, and the sequence Gly-Ser-$(His)_6$ added to the C-terminus, may be expressed in CHO cells. These cells primarily secrete a processed mixture of TACE, about half beginning with Val212 and about half with Arg215. The mixture is purified as described above. The purified TACE polypeptide, with the added binding partner, is stored in a buffer as described above.

The TACE polypeptide and binding partner are co-crystallized. The TACE/binding partner solution, at a polypeptide concentration of about 5 mg/mL to about 12 mg/mL in a TACE buffer described above, is mixed with a suitable crystallization buffer and crystallized using a suitable crystallization technique, for example drop vapor diffusion. Suitable crystallization buffers, for example, include: 0.1 M Na Acetate pH 5.3, 0.2 M $CaCl_2$, 30% v/v Ethanol; 0.1 M Na Citrate pH 5.0, 40% v/v Ethanol; 0.1 M Na Citrate pH 8.7, 20% w/v PEG 4000, 20% v/v Isopropanol; and 0.1 M Na Citrate pH 5.4, 20% w/v PEG 4000, 20% v/v

projecting from the molecular surface (top left in Figs. 1 and 2). A bulged-out loop links sV with the "active-site helix" hC, which is located in the center of the molecule and stops abruptly at the strictly conserved Gly412, where the chain kinks down to build the lower subdomain.

of SEQ ID NO:8

The C-terminal chain comprising the last 61 TCD residues (Fig. 3) first forms three short straight almost perpendicularly arranged segments linked by two "narrow" supertwisted loops, returns via the tight "Met-turn" Tyr433-Val434-Met435-Tyr436 back to the surface where it kinks at Pro437 to form the Pro437-Ile438- Ala439 outer "wall" of the S1' crevice, approaches in a wide loop the C-terminal α-helix hD and runs through it, and ends up on the molecular "back" surface close to the N-terminus, with the last defined residues Arg473-Ser474 fixed via hydrogen bonds to the main molecular body. Via Cys423-Cys453, the first of the two "narrow" loops is disulfide-linked with the N-terminus of helix hD, whose C-terminal end in turn is clamped to the "ear-like" sIV-sV linker peptide through Cys365-Cys469. Spatially adjacent, the third disulfide bridge of TCD, Cys225-Cys333, connects the N-terminal parts of β-strands sI and sIII. In the intact TACE molecule, four residues downstream of Ser474 would reside Cys478, which is already integral part of the compact elongated disintegrin domain (Saudek *et al.*, "Three-dimensional structure of echistatin, the smallest active RGD protein" *Biochem.* 30, 7369-7372 (1991)). Considering Ser474 and this Cys478 as pivot points of their respective domains, the three residue linker would allow relatively unconstrained docking of the disintegrin domain to the "left" surface side of the catalytic domain.

confusing if SEQ ID NO:8 which only has 477 residues

The active-site cleft of TACE (Fig. 2a) is relatively flat on the left hand (non-primed) side, but becomes notched towards the right. The catalytic zinc residing in its center is penta-coordinated by the three imidazole N∈2 atoms of His405, His409 and His415 (provided by the active-site helix and the following "descending" chain comprising the conserved zinc binding consensus motif

of SEQ ID NO:8

corrected in substitute

HEXXHXXGXXH), and by the carbonyl and the hydroxyl oxygen of the hydroxamic acid moiety of the inhibiter (see Figs. 1, 2a and 4). This zinc-imidazole ensemble is based on the distal ∈-methyl-sulphur moiety of the strictly conserved Met435, harbored in the Met-turn characteristic for the metzincin clan (Bode *et al.*, "Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'" *FEBS Lett.* 331, 134-140 (1993); Stöcker *et al.*, "The metzincins: Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases" *Protein Sci.* 4, 823-840 (1995)). Both carboxylate oxygens of the "catalytic" Glu406 (which acts as a general base during catalysis (Grams *et al.*, "X-ray structures of human neutrophil collagenase complexed with peptide hydroxamate and peptide thiol inhibitors: Implications for substrate binding and rational drug design" *Eur. J. Biochem.* 228, 830-841 (1995)) squeezed between the zinc-liganding imidazole of His405 and the edge strand, are hydrogen bonded to the hydroxyl and the N-H group of the hydroxamic acid (see Fig.4). To the right of the catalytic zinc opens the deep S1' pocket, which, besides the S1' wall-forming segment (bottom, Figs. 1 and 2a), is bordered by the side chains of His405 and Glu406 (left), the sIV main chain and the Leu345 side chain (top), and the side chains of Val440 (back) and Ala439 (right). To the right of Ala439 opens a second (S3') pocket, which inside the molecule merges with the S1' pocket, leaving a small bridge made of the opposing side chains of Ala439 and Leu348 (Fig. 2a).

The (pseudo)peptidic part of the inhibitor binds in an extended geometry to the notched right-hand side of the active-site cleft, mimicking the interaction of the primed residues of a productively bound peptide substrate (Fig. 2a). It runs antiparallel to the upper short bulge Gly346-Thr347-Leu348 and parallel to the S1' wall-forming segment Pro437-Ile438-Ala439, making two and two inter-main chain

hydrogen bonds, respectively. The dominant intermolecular interactions are made by the P1' isobutyl (pseudo-leucyl) side chain of the inhibitor and the essentially hydrophobic S1' pocket, however, is large and accommodates three partially ordered solvent molecules in addition. The P2' t-butyl side chain extends away from the enzyme, but nestles to the hydrophobic canopy above formed by the enzyme's bulge. The P3' Ala side chain points into the large negatively charged S3' pocket, but is too short to make favorable contacts. The C-terminal diaminoethyl group has different conformations in the four molecules.

The P1' to P3' segment Val77-Arg78-Ser79 of SEQ ID NO:8 of a bound pro-TNFα probably binds in a similar manner, possibly under better matching with the underlying cleft surface; the preceding P3 to P1 residues Ala74-Gln75-Ala76 of SEQ ID NO:8 certainly will align antiparallel to the edge strand, with their side chains extending into the (partially charged) S3 pocket and the (negatively charged) shallow S2 depression, and projecting out of the central cleft, respectively. The primed subsites and surrounding molecular surfaces of TACE are dominated by negative charges, while the non-primed subsites are essentially hydrophobic in nature (Fig. 2a). More distant interactions may be involved in the specificity of TACE for processing pro-TNFα. The 12 residue substrate comprising the pro-TNFα cleavage site can also be split by some of the MMPs, although with less specificity and efficacy (Black *et al.*, "Relaxed specificity of matrix metalloproteinases (MMPs) and TIMP intensity of tumor necrosis factor-α (TNF-α) production suggest the major TNF-α converting enzyme is not an MMP" *Biochem. Biophys. Res. Commun.* 225, 400-405 (1996)). Thus, the preferential processing of the (probably trimeric) (Tang *et al.*, "Human pro-tumor necrosis factor is a homotrimer" *Biochem.* 35, 8216-8225 (1996a); Tang *et al.*, "Length of the linking domain of human pro-tumor necrosis factor determines the cleavage processing" *Biochem.* 35, 8226-8233 (1996b)) membrane-bound pro-TNFα in *vivo* might in part be due to correct assembling, i.e. suitable presentation of the pro-TNFα cleavage segment to the TACE active site in a distinct distance

from the anchoring membrane. Some experimental evidence (Tang *et al.*, *Biochem.* 35, 8216-8225 (1996a); Tang *et al.*, *Biochem.* 35, 8226-8233 (1996b)) suggests that the cleavage site might not be determined by the cleavage sequence alone, but that also the distance to the base of the compact cone formed by the associated C-terminal segments of three TNFα molecules (Jones *et al.*, "Structure of tumor necrosis factor" *Nature* 338, 225-228 (1989)) plays a role. In a productive TACE-proTNFα complex, the base of this TNFα-trimer cone (into which the disordered N-termini run up) may be recognized by the "right" side of the TACE catalytic domain (Fig. 2a), with the about 10 residues long spacer favoring the correct placement of the proTNFα Ala76-Val77 scissile peptide bond in the active site of TACE.

The polypeptide topology and in particular the surface presentation of the catalytic zinc prove the catalytic domain of TACE to be a typical metzincin. (Bode *et al.*, "Astacins, serralysins, snake venom and matmrix metalloproteinases exhibit identical zinc binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'" *FEBS lett.* 331, 134-140 (1993); Stöcker *et al.*, "The metzincins: Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases" *Protein Sci.* 4, 823-840 (1995)) A superposition with the other metzincins shows, however, that its topology is most similar to that of the catalytic domain of snake venom metalloproteinases such as adamalysin II (Fig. 5). (Gomis-Rüth *et al.*, "First structure of a snake venom metalloproteinase: prototype for matrix metalloproteinases/collagenases" *EMBO J.* 12, 4151-4157 (1993); Zhang *et al.*, "Structural interaction of natural and synthetic inhibitors with the venom metalloproteinase, atrolysin C (form d)" *Proc. Natl. Acad. Sci. USA* 91, 8447-8451 (1994); Kumasaka *et al.*, "Crystal structure of H2-proteinase from the venom of Trimeresurus flavoviridis" *J. Biochem.* 119, 49-57 (1996)) This close homology is

reflected by the much better simultaneous superposition of the central sheet and the large helices, but in particularly also by a couple of structural features, which TACE shares exclusively with the adamalysins such as: the long helix hB and the preceding multiple-turn loop arranged on top of the β-sheet; the typically arranged and shaped C-terminal helix hC; and the extended C-terminus placed on the backside surface. About 175 of the 263 TACE and 201 adamalysin α-atoms are topologically equivalent (with an rms deviation of 1.3 Å, 39 of which have identical side chains (Fig. 3). These numbers are close to those obtained from a comparison of members within the different metzincin families. (Stöcker *et al.*, *supra*) In addition, detailed structural features prove the close relationship of TACE to the adamalysins: a more conserved core structure; the loosely arranged N-terminus; the characteristic Asp416 (directly following the zinc binding consensus motif, Fig. 3) involved in identical intramolecular hydrogen bond interactions; the adjacent disulfide bridge Cys423-Cys453 of SEQ ID NO:8 linking the first narrow loop to the C-terminal helix hD (which TACE does not share with adamalysin II, but with the H2-proteinase from the snake venom of T. flavoviridis) (Kumasaka *et al.*, *supra*); disulfide bridge Cys365-Cys469 of SEQ ID NO:8 connecting the sIV-sV linker with the C-terminal helix hD; a similarly shaped active-site cleft, with particularly strong similarities in the SI' pocket and other primed subsites.

The catalytic domain of TACE (TCD) also differs from adamalysin II in several respects: with 263 residues, its chain is much longer; most of the additional residues of TACE are clustered giving rise to a more projecting hA-sII turn, to the two surface protuberances of the multiple-turn loop, to the two "ears" of the sIV-sV linker, and to a more bulged-out sV-hC connector (see Figs. 3 and 5); lack of a calcium binding site but presence of a third disulfide bridge Cys225-Cys333 of SEQ ID NO:8 in TACE, both elements serving, however, for the same function namely to clamp the N- terminal chain to strand sII; the quite deep S3' pocket of TACE which merges

zinc. Further exemplary compounds include compounds are designed to form a hydrogen bond with Leu348 or Gly349 of TACE (SEQ ID NO:8), (ii) introduce a non-polar group which occupies the S1' pocket of TACE, (iii) introduce a group which lies within the channel joining S1' - S3' pockets of TACE and which makes appropriate van der Waal contact with the channel, and (iv) form a hydrogen bond with Leu348 or Gly349 of SEQ ID NO:8 on the backbone amide groups of TNF-α-converting enzyme, or (v) any combination of the above.

Computer-Readable Medium

The present invention also relates to a computer-readable medium having recorded thereon the x-ray diffraction structure coordinates of a crystalline TACE polypeptide. The computer-readable media of the invention are useful for storage, transfer, and use with software of the TACE structural coordinates. The computer readable medium may be any suitable data storage material, including, but not limited to, a floppy disc, a hard disc, computer-type Random Access Memory, Read-Only Memory flash memory, CD-ROM, recordable and rewritable CDs, recordable and rewritable DVDs, magnetic-optical disk, ZIP drive, JAZ drive, Syquist drive, digital tape drive, or the like. Other suitable media will be known to those of skill in the art.

In one embodiment, the computer readable medium comprises the coordinates of Table 1 or a substantial portion thereof. The computer-readable medium may be used in conjunction with a machine programmed with instructions for using the data recorded on the medium, such as a computer loaded with one or more programs identified throughout the specification, to display a graphical, three-dimensional representation of a TACE polypeptide, or any part thereof.

invention, it should be understood that the following examples are illustrative and not limiting.

Example 1 - TACE Polypeptide Expression, Isolation, and Purification

A cDNA encoding the signal peptide, pro and catalytic domains of TACE, (SEQ ID NO:8) amino acid residues 1-477, as disclosed in Black et al., "A Metalloproteinase disintigrin that releases tumour-necrosis factor-α from cells," *Nature* 385: 729-733 (Feb. 1997), with Ser266 changed to Ala, Asn452 changed to Gln and the sequence Gly-Ser-$(His)_6$ added to the C-terminus, was inserted into an expression vector for CHO cells. The TACE polypeptide was expressed in CHO cells and a mixture of the TACE polypeptide beginning either with Val212 or Arg215 was secreted. The cells were cultured in the drug, methotrexate, which kills those cells that did not incorporate the vector.

The expressed TACE polypeptide was then purified. Purification started with 5 liters of the medium containing the expressed TACE polypeptide. The medium was concentrated to about 200 mL with a Millipore 10K cut-off, 1 ft^2 TFF diafiltration unit. The pumping rate was 50-100 mL/min. Two liters of a buffer solution of 20 mM Tris (pH 7.5) and 300 mM NaCl (Buffer E) was then added to the sample.

The sample was reconcentrated as described above and diluted a second time with 2 liters of Buffer E, reconcentrated again, diluted a third time with 2 liters of Buffer E, and reconcentrated to about 100 mL. The sample retained in the diafiltration unit was recovered by a back-flush. This material was then filtered through a 0.45 μm and was azide added to 0.05%. The filtered sample was stored overnight at 4 °C.

After overnight storage, imidazole was added to the filtered sample to 5 mM from a 200 mM stock in water and $ZnCl_2$ was added to 5 uM from a 1 M stock in

chromatography pool was concentrated to about 1 mL with a 10 K cut-off Amicon Centriprep concentrator.

The inhibitor N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine, 2-(amino)ethyl amide was then added to the purified sample to a concentration of 1 mM. The protein can be stored at 4 °C.

Example 2 - Protein Crystallization

A DNA construct comprising the prodomain and the catalytic domain of human TACE (resides 1-477) was fused to the sequence Gly-Ser-(His)$_6$ to facilitate purification of the protein on a Ni-NTA affinity column. Chinese Hamster Ovary (CHO) were cells used for protein expression. The cells secreted a mixture of mature TACE beginning with either Val212 or Arg215. TACE-containing fractions from the Ni-NTA column were incubated in a buffer containing octylglucoside and the binding partner N-[D,L-[2-(hydroxyaminocarnbonyl)methyl]-4-methyl-pentanoyl)-L-3-(tert-butyl)-glycyl-L-alanine. The final purification step was performed on a gel filtration column. Purified TACE was stored in a buffer containing 10 mM Tris/HCL pH 7.5, 100 mM NaCl, 10% glycerol and 1 mM of inhibitor (TACE buffer).

Crystallization experiments were set up at a TACE concentration of approximately 5 mg/mL by mixing TACE (in TACE buffer) in a 1:1 ratio with the crystallization buffers listed below and using the sitting drop vapor diffusion technique. The experiments were performed in duplicate and incubated either at about 4°C or at 20°C. Crystalline precipitate was obtained at 20°C in the following crystallization buffers:

Buffer A) 0.1 M Na Acetate pH 5.3, 0.2 M $CaCl_2$, 30% v/v Ethanol

Buffer B) 0.1 M Na Citrate pH 5.0, 40% v/v Ethanol

Buffer C) 0.1 M Na Citrate pH 8.7, 20% w/v PEG 4000, 20% v/v Isopropanol

Four independent TACE molecules form the periodic arrangement.

Molecules 1 and 2, and 3 and 4 are defined from Asp219 and Met221, respectively, to Ser474. of SEQ ID NO:8

Example 4 - X-ray Diffraction

Anomalous dispersion diffraction data to 2.0 Å were collected with a MAR345 imaging plate scanner at 100 K on the wiggler beamline of DORIS (DESY, Hamburg, Germany), using monochromatic X-ray radiation of maximal f' (1.2797 Å) and minimal f' (1.2804 Å) at the K absorption edge of zinc and at a remote wavelength (1.060 Å). These data were evaluated and scanned using DENZO/SCALEPACK, yielding 77,653 independent reflections (96.9% completeness, R-merge 0.031).

The structure coordinates obtained are reproduced in Table 1.